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Photodynamic Therapy for Rheumatoid Arthritis?

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Background and Objective: **The only early surgical therapy of rheumatoid arthritis is synovectomy. But even an arthroscopic synovectomy is restricted to more or less big joints. It has been shown recently that for smaller joints a laser synovectomy is possible but more time-consuming than with mechanical instruments. An alternative method may be photodynamic therapy.**
Study Design/Materials and Methods: **In this study, possible photodynamic effects of Chloroquine, Methotrexate, Piroxicam, and Sodium Morrhuate were examined using a cell culture model of human synovial fibroblasts from patients having rheumatoid arthritis.**
Results: **Incubation with Chloroquine or Methotrexate and subsequent laser irradiation at a wavelength of 351 nm resulted in an at least twentifold enhanced cytotoxicity.**
Conclusion: **Both substances therefore may serve for a photodynamic therapy of rheumatoid arthritis. Lasers Surg. Med. 21: 359–364, 1997.** **© 1997 Wiley-Liss, Inc.**

Key words: Cell culture; excimer laser; orthopedic surgery; synovectomy

INTRODUCTION

In Western countries, between 1% and 3% of the population suffer from rheumatoid arthritis. In spite of the success of modern disease-modifying drugs, the natural course of rheumatoid arthritis, which leads to progressive disability, cannot be stopped. In orthopedic surgery, most therapies, e.g., arthroplasty, total joint replacement, and arthrodesis, are restricted to later stages of the disease [1,2]. The only "early" therapy in orthopedic surgery is the surgical removal of the inflamed synovium (synovectomy) or its degradation by radionuclides or cytotoxic drugs injected locally (synoviorthesis).

The efficacy of synovectomy is still being discussed. Most authors report a limited duration of 5 years [3]. During the last few years, arthroscopic synovectomy, which is less invasive and immobilising for the patient, has increasingly attracted interest [5]. The arthroscopic technique is limited to more or less big joints. For the smaller joints of hands, fingers, and feet, arthroscopic techniques are not yet established. Because of the possibility of maximum miniaturization, a laser synovectomy seems to be the perfect solution for those smaller joints. Different laser systems have been tested. The CO_2 laser has a sufficient ablation rate but cannot be used in fluid medium, which is essential for arthroscopy [6,7]. Nd:YAG lasers also have sufficient ablation rates, but thermal side effects cannot be assessed [8,9]. Excimer lasers can be used via an arthroscope, but ablation rates are too low [10]. At present, the best laser system for arthroscopic application seems to be the Ho:YAG, which combines a high ablation rate with acceptable thermal side effects and the possibility of using it in a fluid medium [11–13]. But even with the Ho:YAG laser, synovectomy is more time-consuming than with a mechanical device.

An alternative concept is photodynamic therapy [14]. The principle is that singlet oxygen states are generated when suitable drugs (photosensitizers) are irradiated with the appropriate wavelength [15,16]. Those singlet oxygen states result

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in an oxidation of biomolecules and therefore have a strong cytotoxic potential limited to the area of irradiation. Photodynamic therapies are used experimentally in all fields of surgery. The most experience was gained in the treatment of neoplasms of the urinary tract [17]. Classical photosensitizers are derivatives of hematoporphyrin, but many conventional drugs also have a photodynamic potential [18]. Therefore, another idea is to use the photodynamic potential of drugs, which are given to a patient in any case [19,20]. In the case of rheumatoid arthritis, nonsteroidal antiinflammatory drugs, disease-modifying drugs, and cytostatic drugs may be used [21]. Therefore, the aim of our study was to investigate if some of these drugs can serve as photodynamic therapy for rheumatoid arthritis.

MATERIALS AND METHODS

Drugs

The following substances were examined in this study: Chloroquine, Methotrexate, Piroxicam, and Sodium Morrhuate.

Laser System

A Lambda Physik LPX 325 ICC at a wavelength of 351 nm was used. Pulse duration was 25 ns and a frequency of 100 Hz was selected. The original beam dimensions were 30 mm × 10 mm. After variable diminuation, the beam was defocussed by two lenses (f1 = 100, f2 = 1000) separated by a distance of 1,100 mm. After the second lens, the beam had a circular profile with a diameter of 5 cm and was deflected in a 90° angle onto the cell cultures by a plano mirror. Energy output was measured before and after irradiation by a Gentech joulemeter. Each culture well was irradiated individually. Culture wells were positioned in the center of the beam on a regulated precision heating plate at a temperature of 37°C.

Cell Cultures

Human fibroblasts were taken from patients with rheumatoid arthritis undergoing synovectomy with informed consent. Synovial tissue was scissored under sterile conditions and digested with a dilution of collagenase and DNAse under continuous swirling for 4 hours. After Ficoll density gradient centrifugation cells of the upper phase were collected and incubated in 75 cm^2 culture flasks for 24 hours. After removal of the supernatants, a continuous layer of cells had formed consisting of >95% fibroblasts. Cells were cultivated in a humidified 5% CO_2 atmosphere at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum. The medium was changed twice weekly.

Cytotoxicity

Of drugs. All tests were performed in triplicate and results were confirmed twice. Between 0.9 and 1.3 × 10^4 cells were transferred to petridishes (diameter 30 mm, medium volume 1.5 ml, resulting average thickness of medium 2.1 mm) using a rubber policeman. After 24 hours, the cells were counted again. For the cytotoxic effect, the cells were exposed to different concentrations of each drug varied in a dilution series. After 24 hours, the cells were cultivated in standard medium again. Cell numbers were counted every 12 hours, and results were depicted in growth curves.

Of laser irradiation. For the experiments medium was replaced by phosphate buffer that was shown to absorb <2% of laser energy at a thickness of 1 cm at 351 nm. Irradiation was performed as described above. Energy density and irradiation time were varied, whereas negative controls were subjected to similar culture conditions. After irradiation phosphate buffer was replaced by medium and cells were counted again. Cell growth was depicted in growth curves.

Photodynamic Effect

Cells were incubated with sublethal concentrations of each substance for 24 hours. After replacing the medium by phosphate buffer, cultures were irradiated with an energy density of 1 mJ/cm^2/pulse for 1 minute. Controls were grown under equal conditions: cells incubated with drug but not irradiated; cells irradiated without prior incubation with drug; cells irradiated and afterwards incubated with drug and cells neither incubated with drug nor irradiated. Cell numbers were examined as described above.

Statistical Methods

Cell numbers after 48 hours were compared using a Chi-square test. A probability <5% was called significant.

RESULTS

Cytotoxicity

Of drugs. Synovial fibroblasts from patients having rheumatoid arthritis were treated either with Chloroquine, Methotrexate, Piroxi-

MTX

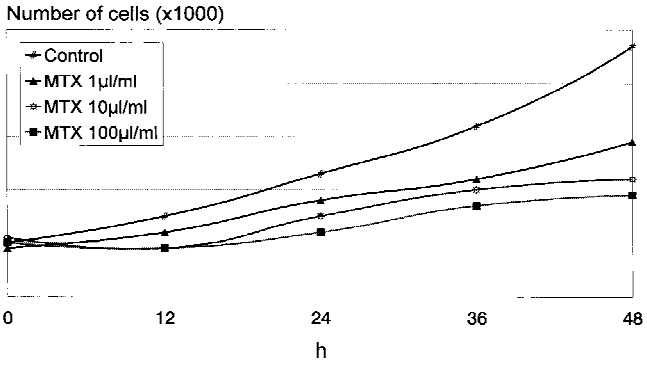

Fig. 1. Growth curves of human synovial fibroblasts from a patient having rheumatoid arthritis incubated with different dilutions of Methotrexate. On the y-axis the number of cells and on the x-axis the observation time are depicted. A reduction of cell growth to ~75% of the untreated control was found at a dilution of 1 µl/ml.

Laser

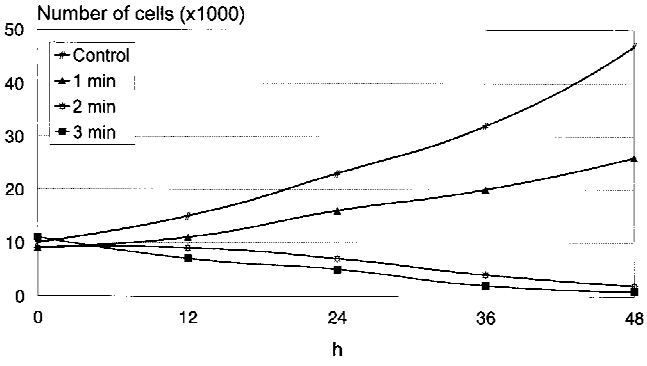

Fig. 2. Growth of human fibroblasts from a patient having rheumatoid arthritis when irradiated with laser at 351 nm, 100 Hz, and 1 mJ/cm^2/pulse. On the y-axis the number of cells and on the x-axis the observation time is depicted. Curves are shown for an irradiation time of 1, 2, and 3 minutes. A reduction of cell growth to ~75% of the untreated control was observed after an irradiation time of 1 minute.

cam, or Sodium Morrhuate, and cell growth was evaluated. Depression of cell growth was dependent on the concentration of each drug. Results for different concentrations of Methotrexate are shown in Figure 1. A sublethal concentration with a cell survival of ~75% was found at a concentration of 1 µl/ml. Sublethal concentrations were found for Chloroquine at 0.02 mg/ml, for Piroxicam at 0.02 mg/ml, and for Sodium Morrhuate at 0.25 µl/ml.

Of laser irradiation. Cultures were irradiated at 351 nm with a frequency of 100 Hz at varying energy densities and irradiation times. Cell survival was dependent on the total energy density. When an energy density of 1 mJ/cm^2/pulse was chosen, a cell growth depressed to ~75% of the untreated control was found after an irradiation time of 1 minute. Figure 2 shows the effect of irradiation times between 1 and 3 minutes on cell growth at an energy density of 1 mJ/cm^2/pulse.

Photodynamic Effect

For the examination of possible photodynamic effects, fibroblast cultures were incubated with a sublethal dilution of each drug and irradiated at 351 nm with 1 mJ/cm^2/pulse for 1 minute. A photodynamic effect was considered when the cytotoxic effect exceeded the sum of laser irradiation and drug treatment alone. When cells were incubated with Piroxicam and Sodium Morruhate, the cytotoxic effect was equivalent to the total of drug treatment alone plus laser irradiation alone. The same cytotoxic effect was observed when cells were first irradiated and afterward incubated with both substances.

In contrast after preincubation with Chloroquine and Methotrexate and irradiation, an enhanced cytotoxicity was found that was at least twentifold higher than the sum of drug treatment alone and laser irradiation alone. Differences were found to be significant for both substances ($P < 0.01$ for Methotrexate, $P < 0.02$ for Chloroquine). When cultures were irradiated with laser and afterward incubated with Chloroquine or Methotrexate, the cytotoxic effect was equivalent to the sum of drug treatment alone and laser irradiation alone. Figure 3 shows the photodynamic effect of incubation with Methotrexate and irradiation at 351 nm. A similar effect was observed at a concentration of 0.02 mg/ml of Chloroquine (Fig. 4).

Histologic characteristics of the cells after preincubation with Chloroquine or Methotrexate and subsequent laser irradiation at 351 nm were a ballooned cytoplasm and the loss of fibroblast appearance to a more rounded morphology. Nuclei showed signs of nucleolysis or nucleorrhexis. After 24 hours most of these cells had detached from the culture surface. Trypan blue staining proved that these cells were not able to keep the dye out of their cytoplasm.

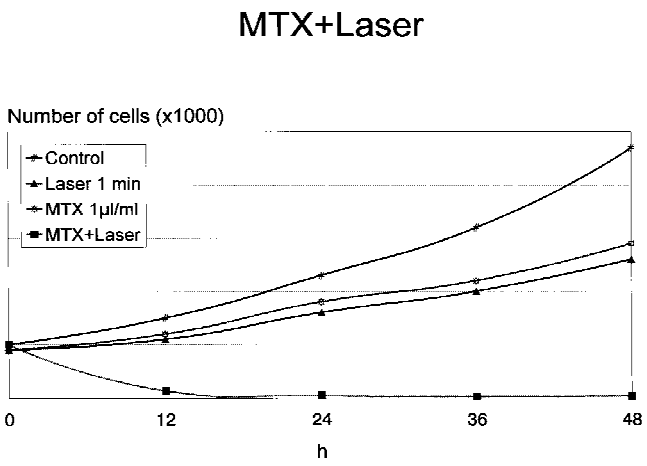

Fig. 3. Photodynamic effect of preincubation with Methotrexate and irradiation at 351 nm. On the y-axis the number of cells and on the x-axis the observation time are depicted. The first curve shows the negative control, the second the effect of a 1 µg/ml dilution of Methotrexate alone, and the third the effect of an irradiation with 100 Hz and 1 mJ/cm^2/pulse for 1 minute alone. In contrast, irradiation with laser after preincubation with Methotrexate results in an at least twentifold higher cytotoxicity than the sum of single effects ($P < 0.01$).

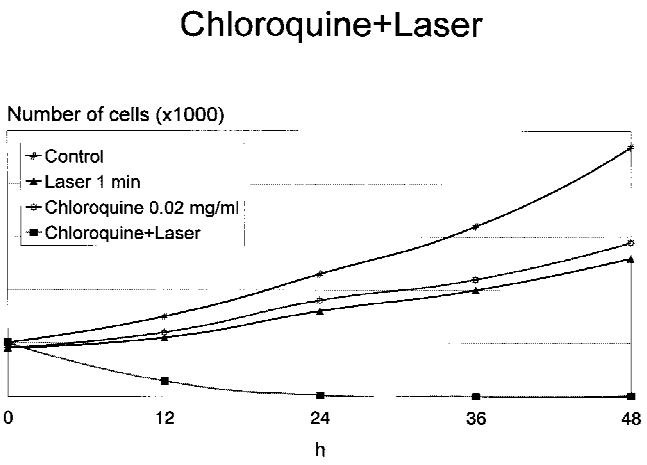

Fig. 4. Photodynamic effect of preincubation with Chloroquine and irradiation at 351 nm. On the y-axis the number of cells and on the x-axis the observation time are depicted. The first curve shows the negative control, the second the effect at a concentration of 0.02 mg/ml of Chloroquine alone, and the third the effect of an irradiation with 100 Hz and 1 mJ/cm^2/pulse for 1 minute alone. In contrast, irradiation with laser after preincubation with Chloroquine results in an at least twentifold higher cytotoxicity than the sum of single effects ($P < 0.02$).

DISCUSSION

The therapeutical situation for rheumatoid arthritis is still unsatisfactory [1,2]. Therefore, the aim of this study was to examine the possibility of a photodynamic therapy in a cell culture model.

Human synovial fibroblast cells were chosen because they represent the majority of cells in the rheumatoid synovium. In the broadened synovial layer, 60–80% of the cells are type II and III cells, which means they have a fibroblast morphology [22]. Beside the fibroblast cells of the rheumatoid pannus tissue, the lymphoplasmacellular infiltration surely also is a main target of the photodynamic therapy; however, fibroblastoid elements make up the main part of the inflamed tissue.

An alternative concept of photodynamic therapy is to use the phototoxicity of conventional drugs. It has been shown previously for Epirubicin that its cytotoxicity can be enhanced by irradiation at 351 nm [19]. Like Epirubicin, the substances used in this study have an absorption peak at 351 nm. Chloroquine, Methotrexate, and Piroxicam are administered systemically, whereas Sodium Morrhuate is injected intra-articularly.

In Germany, all drugs are approved for therapeutical use. Their kinetics, side effects, and long-term cytotoxicity are well documented [23]. When fibroblast cultures were incubated with these drugs and irradiated at 351 nm, an enhanced cytotoxicity was found for Chloroquine and Methotrexate. The cytotoxic effect was not additive because it was at least twentifold higher than the sum of drug treatment and laser irradiation alone. Actually, the present examination did not explicitly show that this enhanced phototoxicity is due to a photodynamic effect (i.e., that singlet oxygen is produced). Yet, the photodynamic effects of Chloroquine and Methotrexate already have been described. In the case of Chloroquine a directly photosensitizing effect was found after irradiation with UV-light [24], whereas Methotrexate as a substance was not active photodynamically. However, it has been shown that after UV-irradiation of Methotrexate, photoproducts are produced that exactly match that of hematoporphyrin derivative [25]. For these photoproducts a generation of singlet oxygen could be proved [25]. Therefore, we conclude that the enhanced cytotoxicity of Chloroquine and Methotrexate is due to their photodynamic effects described previously. In combination with the wavelength 351 nm, both substances may serve for a photodynamic therapy of rheumatoid arthritis. An accumulation of both substances can be assumed because of the high grade of vascularization of the inflamed tissue. If local concentrations after systemical application are not sufficient an intraarticular injection is possible.

The wavelength of 351 nm has not been used in orthopedic procedures yet. Recent studies show that ablation thresholds for bone, vascular, and lens tissue are far beyond the energy fluence of 1 mJ/cm^2/pulse used in this study [26–28]. Therefore, within a joint a photomechanical effect of this fluence could result in a superficial vacuolization of a few μm depth [26]. In our experiments the photomechanical contribution to cell death initially was 15% at best, but a longer observation time showed only a lag in growth (Fig. 3). The exact photomechanical effect of 351 nm within a joint remains to be elucidated in an in vivo study, but the available data suggest that it will be only of minor clinical significance.

The main advantage of a photodynamic therapy for rheumatoid arthritis is its minimal invasive character. It has been shown previously in angioplasty that 351 nm can be delivered via silica fibers [29]. Whereas for large joints, a conventional arthroscopic use is possible, for small joints the use of needlescopes can be taken into consideration that include a working channel for a laser fiber with an outer diameter of <1 mm. In any case, the special fiber tips already in use in photodynamic therapy can be applied by means of an 18-gauge needle. With those small instruments, even a photodynamic therapy of finger and toe joints may be possible. In the case of large joints, a second arthroscopy can be performed in order to remove the debrided tissue, but as the experience with radiosynoviorthesis shows, the natural cleaning process of the joint seems to suffice. For the use of a photodynamic therapy on joints, especially studies aiming at the distribution of light seem to be necessary. The rabbit IgG-induced-arthritis [30] will serve as an animal model to review the results of our cell culture experiments in vivo.

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